Technische Universiteit
Eindhoven
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iGEM TU/e 2014
Biomedical Engineering

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Transformation of vector in NovaBlue

Where innovation starts

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1 Transformation of vector in NB:

- Switch on the water bath and set temperature at 42 °C. Also turn on the heat/shaking-block and set up to 37 °C
- Load a bucket with ice from the ice machine
- Thaw the cells (NB) on ice for ~5 minutes
- Add 1µL of ligation mixture to 20µL NB bacteria. (Leave on ice) . Mix well. Make sure you work near the Bunsen burner flame
  - In our Case OMPX, pEVOL & Pet29A
- Incubate on ice for 5min
- Heat shock the solution (42°C for 30s!!!)
- Return to ice for 2min
- Add 80 µL of SOC solution to the bacteria (Do not return to ice!!)
- Incubate for 60 mins at 37°C and 300 rpm

1.1 Plating of the cells on agar plate (supplemented with antibiotics)

- Take the dried agar plate out of the 37°C incubator
- Label the bottom of the plate with your initials, date, bacterial strain, plasmid type and gene name (mutant)
- Open an agar plate in close proximity of the Bunsen burner flame
- Pipette the cells on the plate
- Spread the cells on the plate using the sterile spatula
- Transfer the agar plate to the 37°C incubator
- Place the plate upside down, closed
- Let the cells grow on the plate overnight